MELANOPHORES INDUCED BY X-RAY COMPARED WITH THOSE EXISTING IN PATTERNS AS SEEN IN CARASSIUS AURATUS[1]

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In a recent publication (Smith, 1932) it was shown that if goldfishes (*Carassius auratus*) were exposed to X-rays, an eruption of corial melanophores occurred varying greatly in intensity in different fishes. In some fishes a general cutaneous melanosis resulted, leading even to death of the fish.

Generally speaking, the X-ray eruption of melanophores is a transient affair, appearing about the fifth day after exposure to X-ray or somewhat later. Newly formed melanophores increase rapidly in numbers to form pigmented areas often visible to the eye. By a process of degeneration, these melanophores disappear, and there results a restoration to normal coloring. At water temperatures of 70° F., depigmentation consumes roughly from two to four weeks, after which the cutaneous regions once more assume a normal color.

X-ray-produced melanophores in the goldfish behave, therefore, in much the same manner as melanophores produced in the same type of fish by trauma or in the healing of wounds or fractures as noted in earlier experiments (Smith, 1931).

It became of interest to learn what relationship, if any, melanophores newly produced from X-raying held toward groups of melanophores already existing in the goldfish in the form of cells massed to form a definite pattern of the body, head, or fins; and further, to note any evidence of degeneration in existing pattern cells following radiation.

Forty-five goldfishes, possessing various black patterns for the most part resembling those seen in Plate I, Fig. 1, were studied after exposure to 7 human erythema units of X-ray (sufficient usually to induce melanophores in the goldfish), and the behavior of melanophores in patterns and melanophores formed by X-raying were compared.

The technique of X-raying, found satisfactory in earlier experiments, was the following. The goldfish, anaesthetised in a solution

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of chloretone 1 to 2000 of water, was removed from this solution and placed on a folded towel directly under the X-ray tube with the entire left side facing directly upward. One unit of human erythema dose consisted of 100 k.p.v., 5 milliampere, 8-inch target-skin distance, no filter, 72 seconds exposure. Seven erythema units involved, therefore, an exposure of 504 seconds. After exposure, the fishes were kept under conditions of ordinary laboratory light in tanks of still water (70°–78° F.) supplied with a current of air. The X-raying was done through the courtesy of Dr. William LaField, Mr. E. E. Furbush of the New Haven Hospital, and Dr. Samuel Atkins of St. Mary's Hospital, Waterbury.

The following are two illustrative experiments, in which X-ray eruptions were intense enough to permit photographing.

Experiment 1. Goldfish, 5 cm. in length from snout to base of tail (Plate I, Fig. 1, Fish *A*, photographed before exposure to X-ray) with markings of massed melanophores on head and fins. This fish received 7 human erythema units of X-ray, exposing entire left side of fish. Eleven days later an active development of melanophores occurred in the exposed surfaces of the fish. Fourteen days after exposure the X-ray eruption appeared to have reached its height and the fish was photographed (Plate I, Fig. 2). Dense masses of X-ray-induced melanophores occupied chiefly the left side of head, body, and fins, and closely encroached upon the periphery of black pigmented patterns. Depigmentation of X-ray-induced melanophores began approximately 3 weeks after exposure. Plate I, Fig. 3, shows fish 27 days after exposure with depigmentation greatly advanced. The head region has cleared, leaving the pattern undisturbed. Complete disappearance of X-ray melanophores was noted on the fifty-fifth day after exposure. Plate I, Fig. 4, was taken on the seventy-ninth day after exposure and shows head pattern practically unchanged. The pigmented markings of the fins showed microscopically no apparent difference from the original arrangement as seen before X-raying.

Although melanophores composing pre-existing patterns remain usually undisturbed by exposure to X-rays in doses of 7 human erythema units, as in the above experiment, in three instances there seemed to be definite evidence of an induced degeneration of melanophores composing a pattern, and the following illustrative experiment describes such a degeneration of pattern following a typical X-ray eruption and depigmentation.

Experiment 2.—Goldfish measuring 6 cm. from tip of snout to base of tail received 7 human erythema units of X-ray, the entire left side of the fish being exposed to the X-ray tube. Plate II, Fig. 1,

is a photograph of this fish 19 days after exposure, showing a marked massing of melanophores, *XR.*, on the left side of the head and operculum, approaching in distribution close to the small head pattern *P.* At the periphery of the head pattern, *P.*, a close intermingling occurred of both pattern cells, *P.*, and X-ray melanophores, *XR.*, seen in higher magnification in Plate II, Fig. 2. Degeneration of melanophore masses induced by X-ray began in the third week, and advanced by the twenty-seventh day to a complete clearing of the head region, the upper part of the operculum alone showing still massed X-ray-induced pigment cells, *XR.* (Plate II, Fig. 3). The head pattern, *P.*, at this time retained the details of its original form except at the caudal tip of the pattern where there was noted some degeneration of melanophores adjacent to an opaque zone (Plate II, Fig. 3, *O–O'*), a point where xanthophores had also disappeared. Plate II, Fig. 4, taken 42 days after exposure, shows the head pattern partly degenerated. Plate II, Fig. 5, 55 days after exposure, shows the head pattern no longer existing; and it was noted that the black pigmented markings on the various fins had disappeared to a very large extent. This particular fish, relatively sensitive to X-ray, showed beside melanophore degeneration, within the first two weeks a degeneration of xanthophores in several areas indicated in the photographs by the letter *O.* Zones of xanthophore degeneration appeared in life as streaky grayish opaque areas, confined chiefly to the left side of the body, which had been directly exposed to X-ray.

Ten fishes failed to give any eruption whatever of melanophores after exposure to seven human erythema units. A month later mechanical injury was produced by crushing the left operculum with an artery clamp. Five days later numerous melanophores developed near the wound in each fish, temperature of water being 76° F. It was believed that in these experimental fishes, X-ray injury was not severe enough to elicit a melanophore reaction.

Explanation of Plate I

Fig. 1, *A*, *B*, *C*, are types of goldfishes employed in these experiments, with black pigment patterns. Fish *A*, photographed on day before X-raying.

Fig. 2, Fish *A.*, 14 days after X-raying showing pigmentation from X-ray-induced melanophores (*XR.*) chiefly on left side or exposed side, encroaching upon region of existing pattern of head, *P.*

Fig. 3, Fish *A.*, 27 days after raying. Depigmentation of X-ray-induced melanophores greatly advanced, eruption showing only at points *XR.*

Fig. 4, Fish *A.*, Photograph shows fish *A* on the seventy-ninth day with head pattern intact. Complete disappearance of X-ray eruption occurred on the fifty-fifth day.

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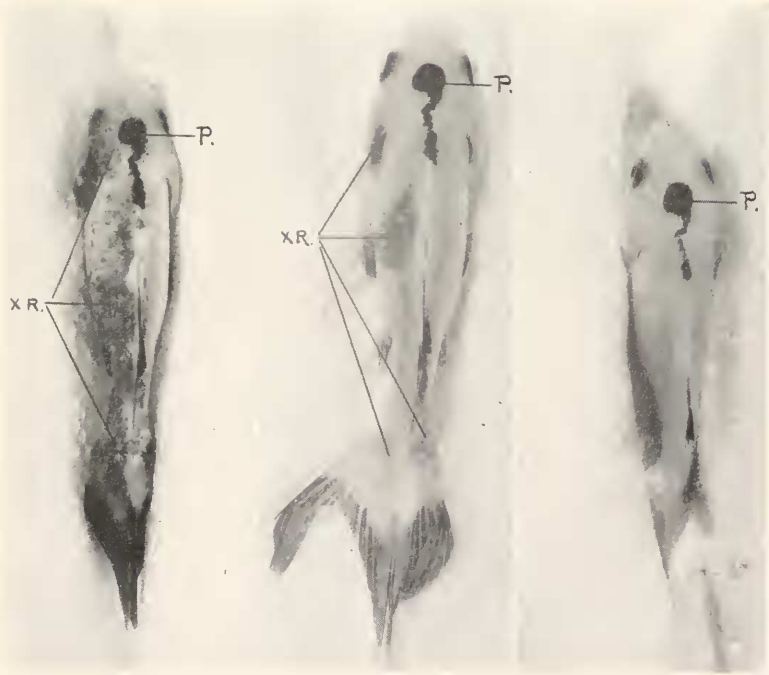

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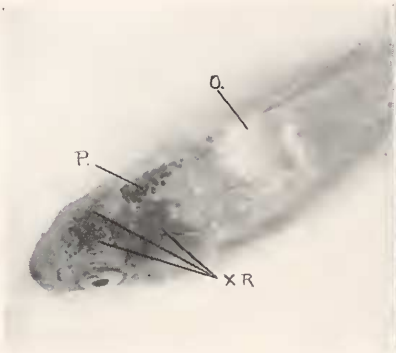
PLATE I



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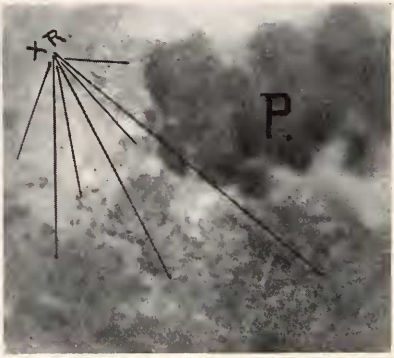

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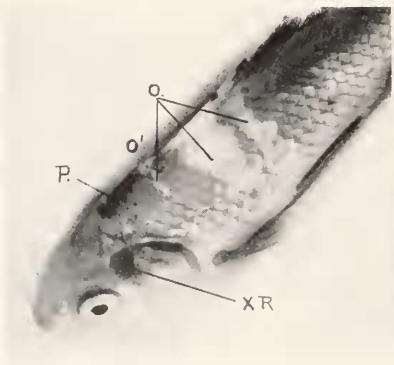

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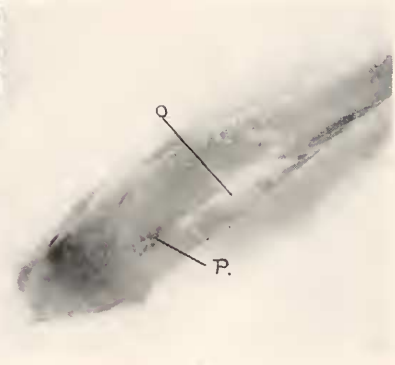

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PLATE II

Comment

Results of experiments indicated that melanophores of already existing black patterns were for the most part not influenced by single doses of X-ray as high as seven human erythema units. At the periphery of such patterns an active development of new melanophores from X-ray exposure might occur here and there, so that pattern melanophores and what may be designated X-ray melanophores grew in close apposition (Plate II, Fig. 2) with much intermingling at the time of the height of the X-ray eruption. Disappearance of the X-ray melanophore eruption left the melanophores of the pattern usually in an intact condition without alterations in the morphology of the cells. Thus, melanophores of two kinds were found to exist in the same fish, reacting differently to the effect of X-ray. On the one hand, melanophores composing existing patterns usually remained stabile, and rarely degenerated. On the other hand, masses of new melanophores evoked by X-ray, pursued a comparatively short life cycle of active growth and early degeneration with complete subsequent depigmentation.

Degeneration of pattern cells was noted definitely only three times among the 45 fishes studied, as illustrated in Experiment 2 cited above. As seen in this experiment, the head pattern slowly degenerated after disappearance of the X-ray-induced melanophore eruption. An extensive though incomplete degeneration of the fin patterns occurred simultaneously with that of the head pattern. In this particular fish, areas of degeneration of xanthophores were noted as well. It is not unlikely that further investigation will show that a dosage somewhat higher than 7 human erythema units will be neces-

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Explanation of Plate II

Fig. 1. Shows goldfish referred to in Experiment 2 with X-ray eruption (*XR.*) 19 days after raying. X-ray eruption (*XR.*) and head pattern (*P.*) are in close apposition. Letter *O* points to an area of xanthophore degeneration.

Fig. 2. A higher magnification of a part of the field in Fig. 1. *XR* points to eruption of massed melanophores induced by X-ray. *P.* represents the massed melanophores forming existing pattern of head. Melanophores of eruption and pattern intermingle at the periphery of pattern *P.* Magnification × 6.

Fig. 3. Same fish as in Fig. 1 with partial depigmentation of X-ray eruption of melanophores 27 days after X-ray exposure. The head region has cleared and shows no eruption of melanophores except at the upper part of the operculum (*XR.*) Pattern *P.* is intact except for slight degeneration posteriorly at *OO'* where xanthophores have also disappeared.

Fig. 4. Same fish 42 days after raying. Head is cleared of X-ray eruption; pattern *P.* is degenerating. Xanthophores have degenerated at point marked *O.*

Fig. 5. Same fish 55 days after raying. Head pattern has degenerated completely. Xanthophore degeneration at point marked *O.*

sary to produce uniformly degeneration of pattern melanophores of this fish, influenced by weight and size of fish.

The melanophores following X-ray exposure may bear a close morphological resemblance to the melanophores of an existing pattern, so that the two types are distinguished often with difficulty. Usually, however, X-ray melanophores look more delicate and smaller than pattern melanophores; their processes are more irregular and reach out into different planes in the tissue spaces. The pattern melanophores appear more flattened as they lie at rest spread out immediately beneath the transparent epithelium. Their borders with processes parallel to the surface appear more sharply circumscribed and deeper pigmented especially when in a somewhat contracted state.

As Fukui (1927) and Goodrich and Hanson (1931) have shown, the young goldfish is normally dark colored as the result of the presence of melanophores. Depigmentation begins irregularly after a few weeks of life and the fish gradually assumes a yellowish, golden color. The extent and completeness of depigmentation determines the pattern of adult conditions, subject probably to still further slow changes in black pigmentation later in life. Melanophores of patterns are probably fully differentiated cells, and closely affiliated with the nervous system as shown by Ballowitz (1893), von Frisch (1911) and other investigators; whereas melanophores evoked by X-ray, or by mechanical injury function perhaps in behalf of body defense and repair, when certain chemical conditions are produced in the corium of goldfishes possessing potential pigment-forming cells.

In the present experiments, areas composed of pattern cells did not seem to develop new X-ray-induced melanophores to any extent except temporarily at the periphery of the pattern. This fact suggests that conditions did not exist in the central parts of the patterns for the development of new pigmented cells, under the conditions of dosage employed, or possibly that the massed flattened pre-existing pattern cells offered enough protection against the effects of X-rays to inhibit the formation of new pigmented cells.

Summary

In the goldfish exposed to X-ray (7 human erythema units) existing patterns remained for the most part intact in the presence of an induced temporary eruption of corial melanophores caused by X-raying. In several fishes, however, a degeneration and disappearance of the patterns, partial or complete, was noted, and this followed after depigmentation of an eruption of X-ray-induced melanophores. X-ray thus produced two effects relative to melanophores, (*a*) an eruption

of new melanophores with a short life cycle, (*b*) occasional degeneration of melanophores in existing patterns.

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